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Title:	Understanding Host Pathogen Interactions: a Foundation for Advanced Therapeutics
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Intended for:	“Advanced Therapeutics” deep dive to be held at the Study Center at LANL on 08/05/2014
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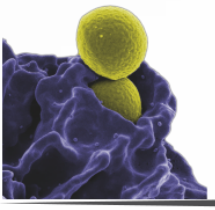

ADVANCED THERAPEUTICS AND VACCINES

Understanding Host Pathogen Interactions: a Foundation for Advanced Therapeutics

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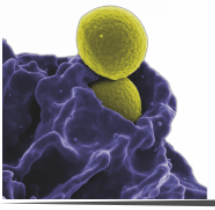

Why “host-pathogen interactions”?

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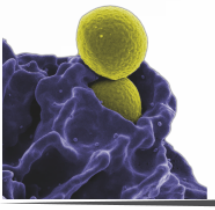
- We need new antibacterial / antiviral compounds
  - New threats
  - Resistance
- In the post-genomics era we can rationally target key host-pathogen pathways
- Potential for broad spectrum
  - Phylogenetically related, emergent threats



Why LANL?

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- Scientific and technological foundation
- Capability for multidisciplinary approach
  - From screening to modeling
- Capability for a sustained effort
- Basic science problem
  - Initially, too hard for “private companies”

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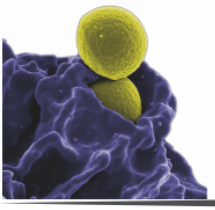
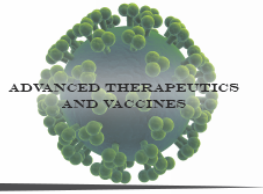
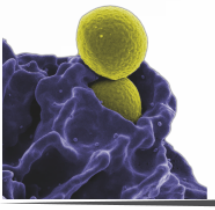


LANL Science / Technology Foundation



- Technology: development and use
  - Automation
  - High throughput
  - State-of-the-art assays
- Science
  - Bacterial pathogenicity
  - Innate immunity
  - Identifying new host-pathogen interactions
  - Modeling: from statistics to bioinformatics to mechanistic models



A few examples

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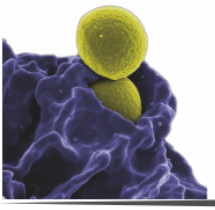

A few examples

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- Influenza

- Burkholderia

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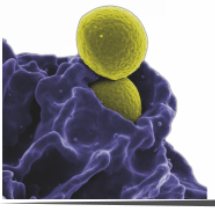

Protein-Protein Interactions

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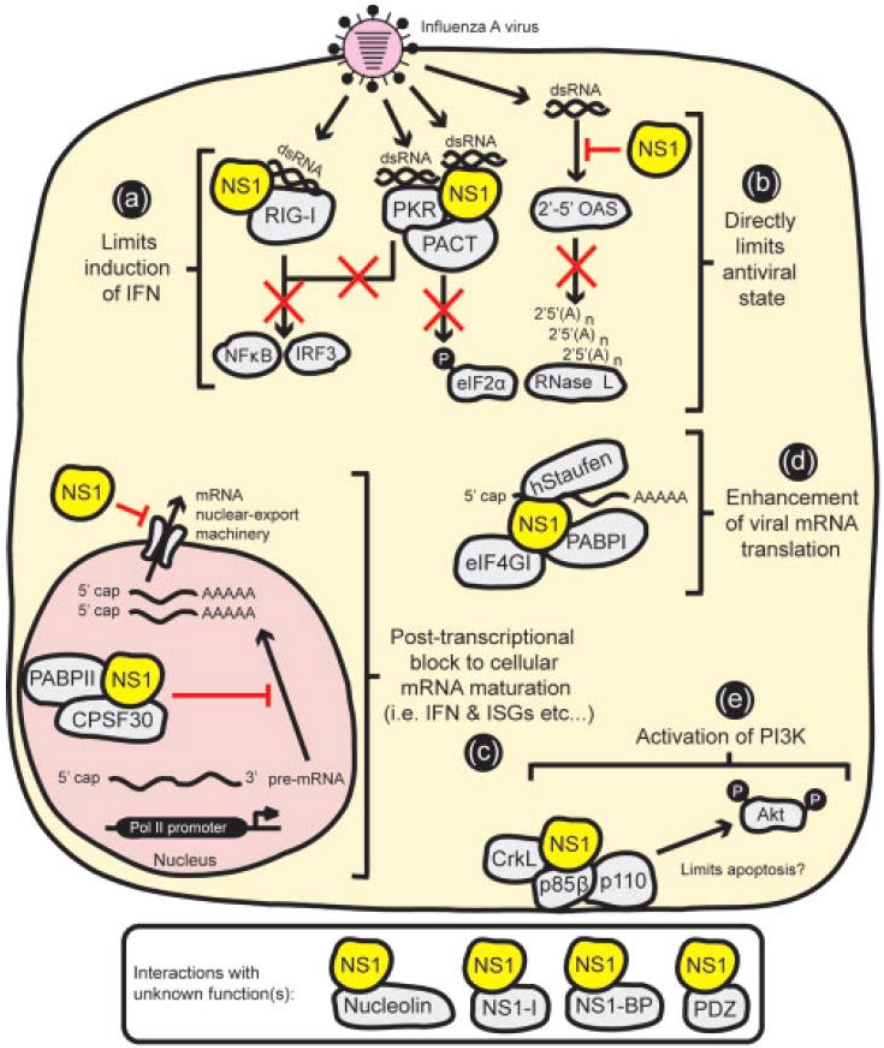
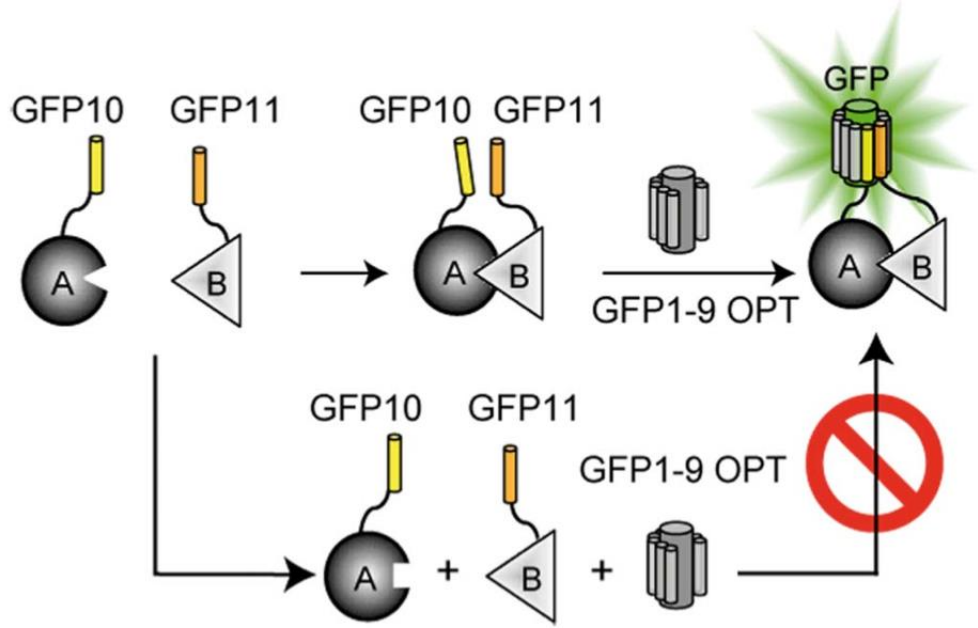
- Are involved in multiple pathogenic pathways
  - Cleavage of pathogen proteins (e.g., influenza)
  - Toxin effects (e.g., *B. anthracis*)
  - Disruption of innate immunity (e.g., influenza, HCV, …)
  - Promote cell entry (e.g., *Burkholderia spp*)

Influenza A NS1 effector/host biology



- NS1 disrupts innate immune responses
- NS1 interacts with multiple host proteins
  - Binds p85β/p110 complex and limits apoptosis

Tracking interactions: *tripartite* GFP

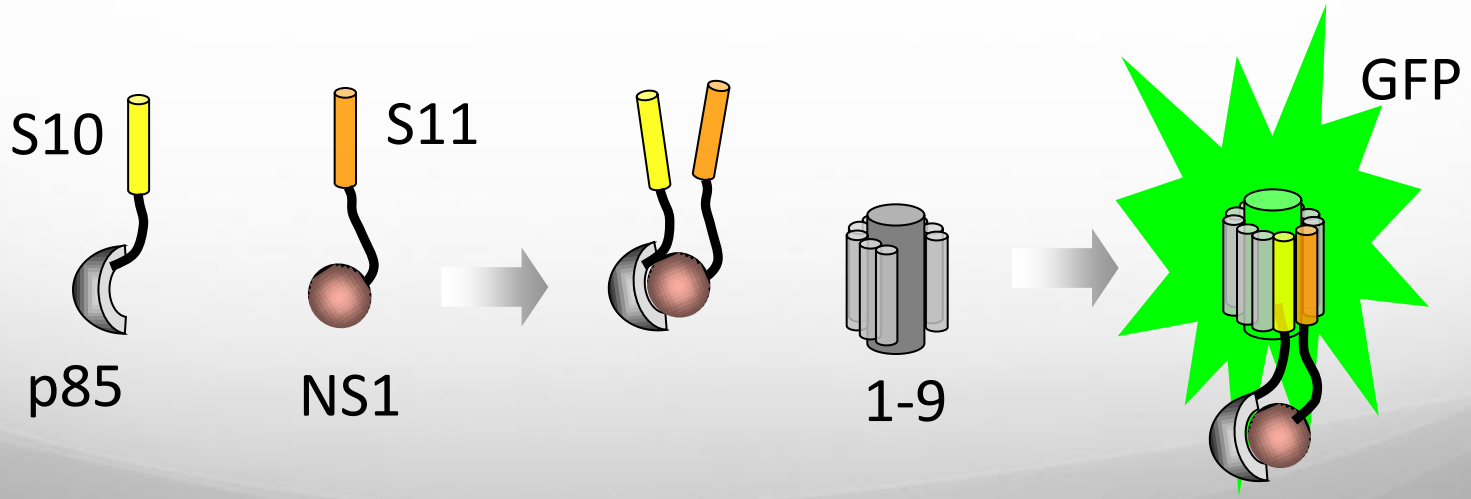


Cabantous S. et. al, 2013 Sci Rep 3, doi:10.1038/srep02854

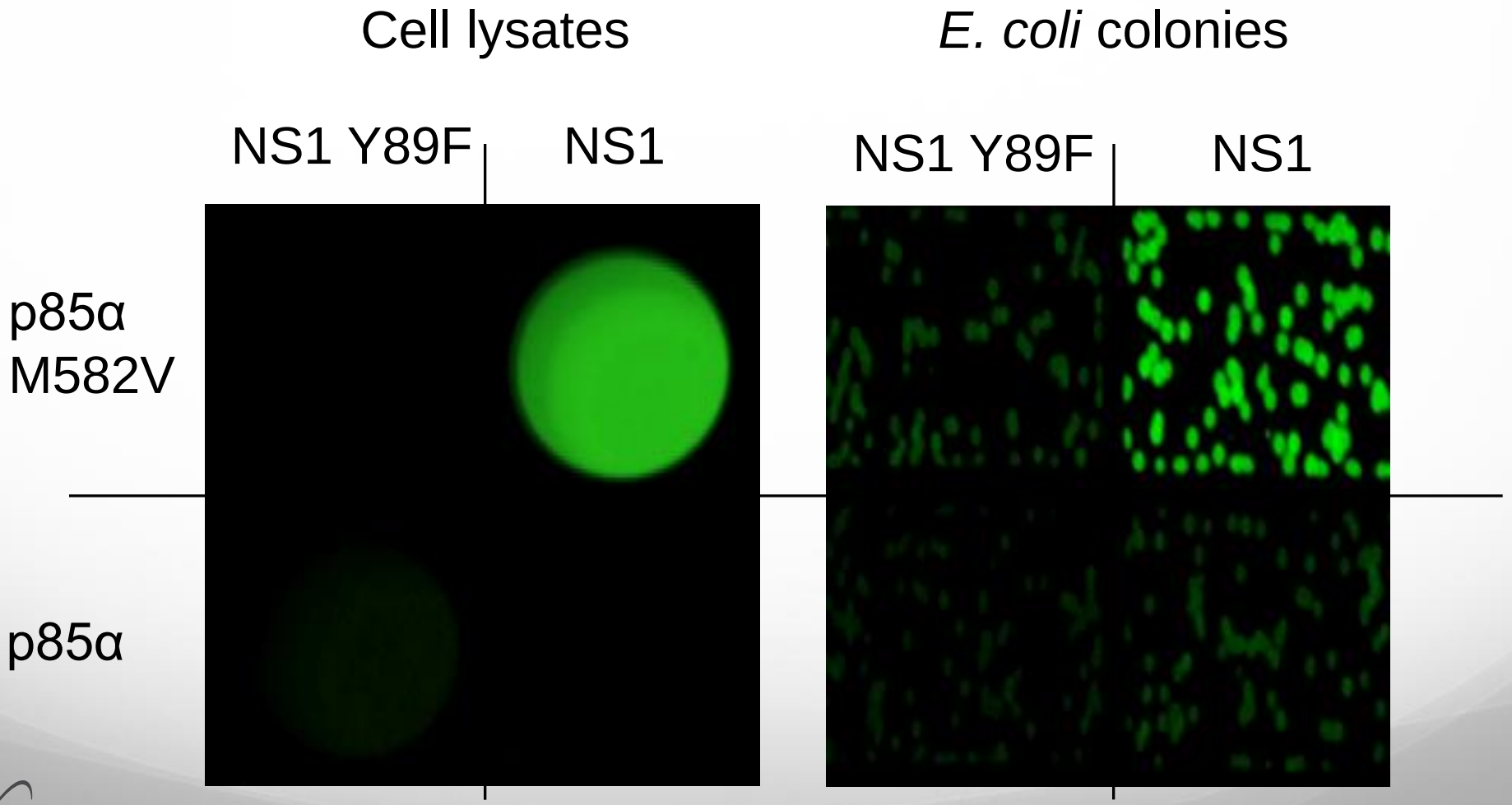
- Attach small 15aa tags to proteins (GFP strands 10 and 11)
- Interaction status is polled by expressing GFP 1-9
- Extensively engineered to not perturb passenger proteins

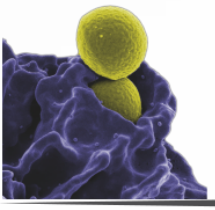
Proof of principle

- NS1 interacts with p85β but not p85α
- A single point mutation (M582V) converts p85α into an NS1 interactor: gain-of-function mutation
- A single point mutation (Y89F) converts NS1 into a non-interactor
- Can we detect these small sequence changes?



GFP based assay





Advantages of tripartite split GFP

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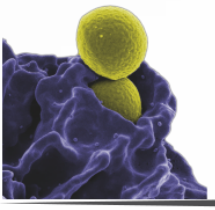
- Small tags: minimize disruption of protein function
- High sensitivity
- Applicable *in vivo*
- Soluble, not temperature dependent
- Spatial resolution



Disrupting host pathogen interactions

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Direct visualization of crucial interactions for pathogen survival / virulence

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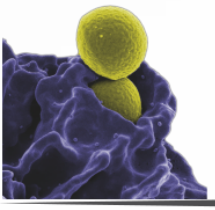


Disrupting host pathogen interactions

ADVANCED THERAPEUTICS AND VACCINES

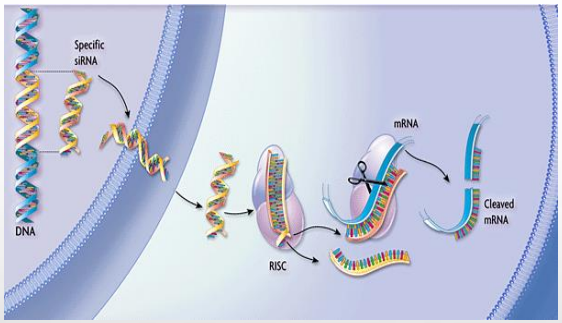
Direct visualization of crucial interactions for pathogen survival / virulence

- Use RNA interference to knock down mRNA expression of target genes
- Target all genes (whole genome) or given pathway
- Measure effect of host mRNA knock down on viability / replication of pathogen

RNAi-based screen of *B. thailandensis* invasion

siRNA library for kinases
Targeting 782 kinases with >2500 siRNA constructs

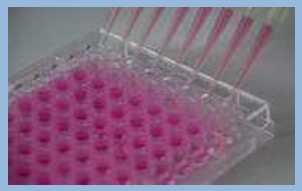
Gene knock-down in the host



Assay strategy

Host cell invasion by GFP-labeled *B. thailandensis*

1) siRNAs (72 hrs)



THP-1

2) B. thailandensis (MOI 10) (20 hrs)

+ chloramphenicol

Read fluorescence in 96 well plates using LSRII flow cytometer

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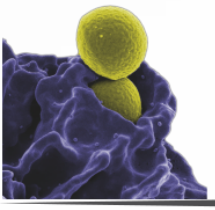

RNAi screen hits

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Actin polymer-ization	Centrosome/ spindle function Mitosis	Ca2+/Cal modulin	PKC / cAMP	PIP	Glyco-genesis	RTK	Adhesion	NF-kB signaling
PAK3 **STK35** PKN3 AKT2 STK11	AURKC, **AURKA** MAST3 UHMK1 STK22D, STK4 **STK38L/NDR1**	**CAMK2B** CAMK2A CAMK1G **CALM1** **CAMKK2**	**PKCη** PRKACB	P101-PI3K ITPKA PIK3R1 **PIP5KIA** PIP5K2B AKT1	**INSRR** PCK1 HK1 HK2 **HK3**	KDR EPHA3 **EPHB3** TYRO3 ERBB3	**PTK7** ABL1	MAP2K7 MAP3K2 **MAP3K11** MAPK6

- Identified ~40 host genes required for entry and intracellular growth of *B. thailandensis*
- Candidate genes classified into multiple interconnected host pathways – some common to infections with other pathogens (e.g. ABL1, AKT1)
- Validate candidate hits using imaging flow cytometry and microscopy

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Validation using Imaging flow cytometry

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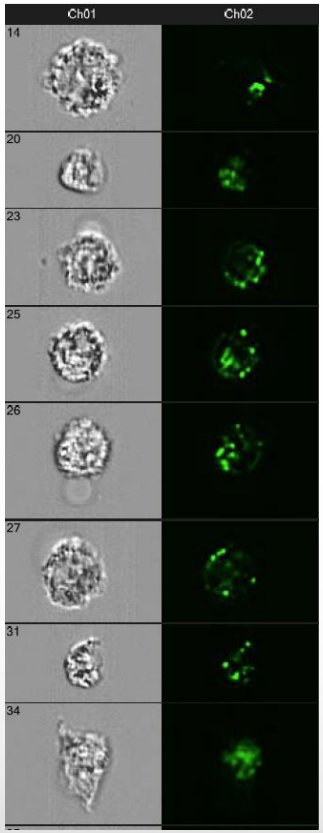
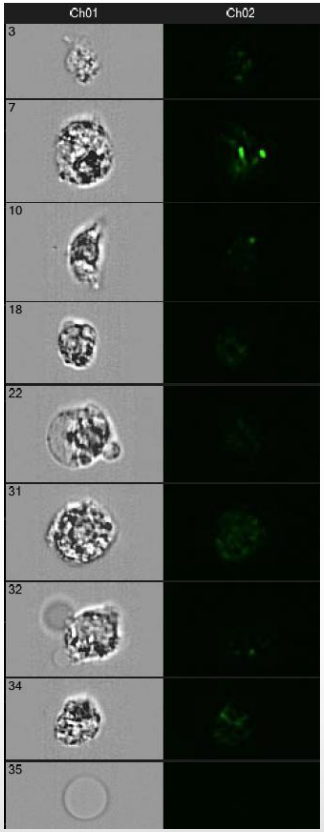
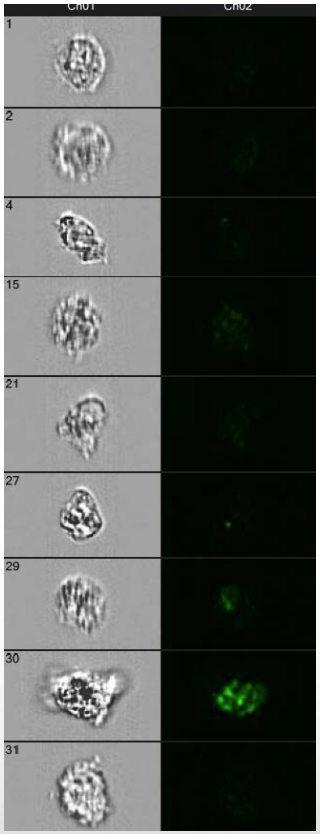
THP-siRNA-Control



THP-siRNA-MAP3K11



THP-siRNA PIP5KIA



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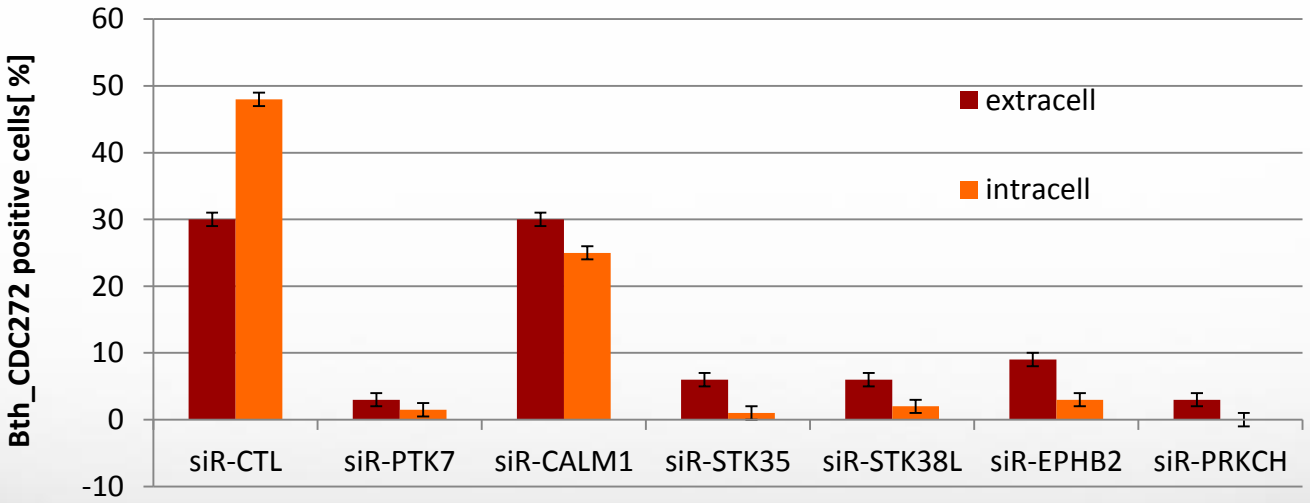
Labeling extra vs intracellular *B. thailandensis*

Intracellular bacteria
Green

Extracellular bacteria
Red



PKCη probably acts through MARCKS

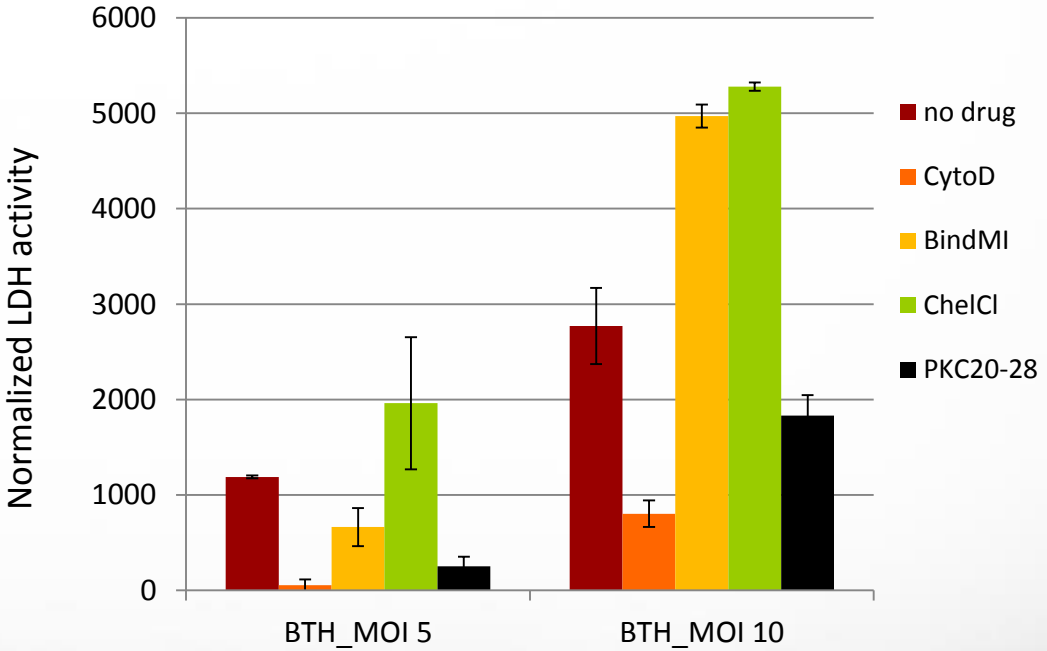
ADVANCED THERAPEUTICS AND VACCINES

PKCη – Protein kinase C-eta

- Regulated by diacylglycerol and phosphatidylserine, but not calcium
- Previously implicated in *Listeria* and *Plasmodium* infection

MARCKS - myristoylated alanine-rich protein kinase C substrate

- An actin filament crosslinking protein
- Cell motility, phagocytosis, membrane trafficking and mitogenesis



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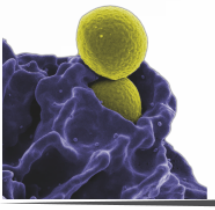

Conclusions

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- We have identified ~40 kinases that regulate *Burkholderia* invasion in the host.
- Small molecules that are FDA approved for cancer treatment can be repurposed / tested as antimicrobials
- We have applied the same ideas to an extracellular pathogen
  - *Yersinia spp*: BMC Microbiology 2013, 13:249

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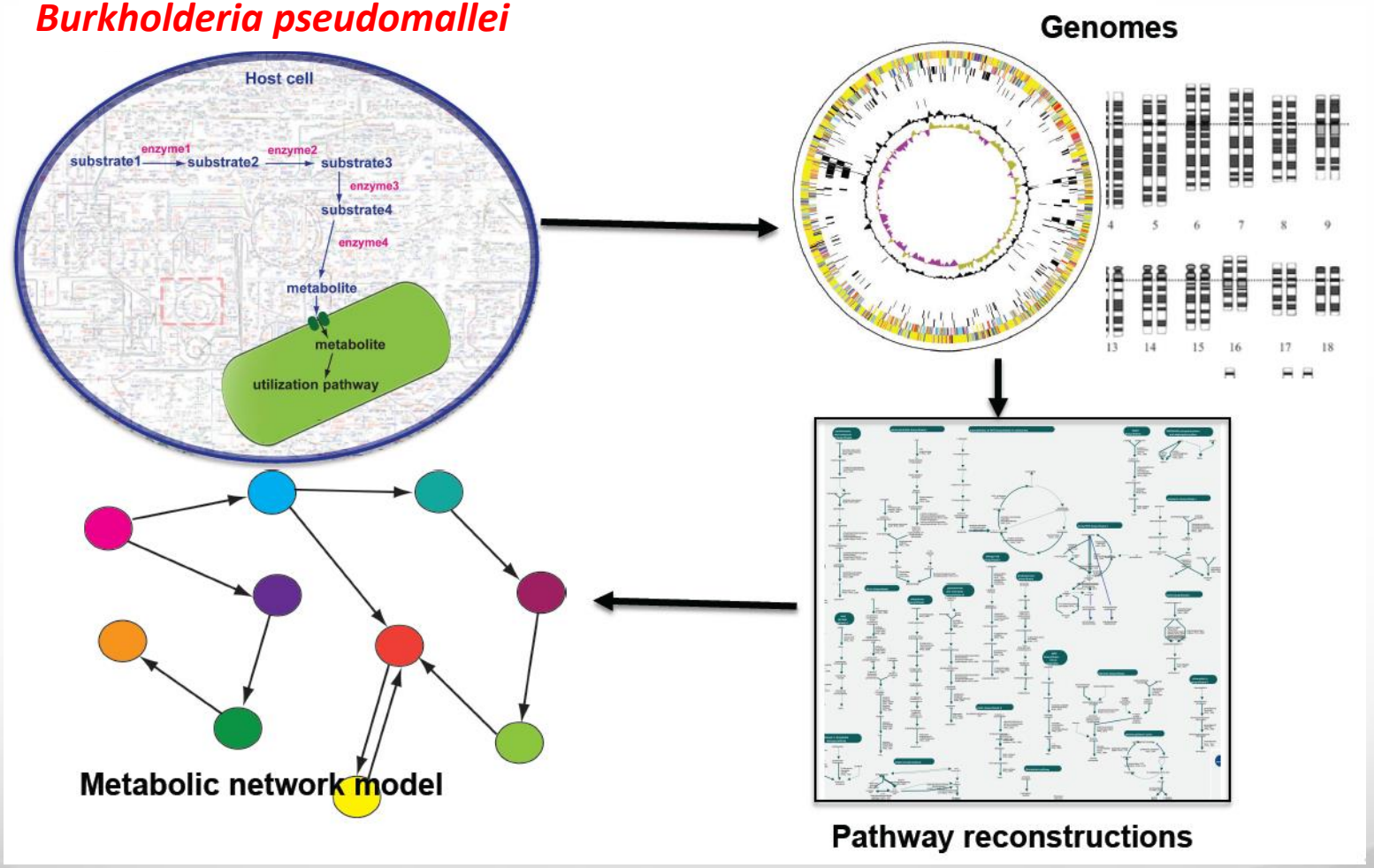
Host-pathogen metabolic interactions

ADVANCED THERAPEUTICS AND VACCINES

Burkholderia pseudomallei



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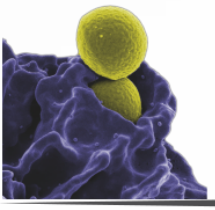

Host response as a pathogen detector

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Different influenza strains will produce individual response profiles

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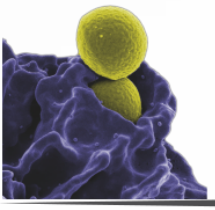


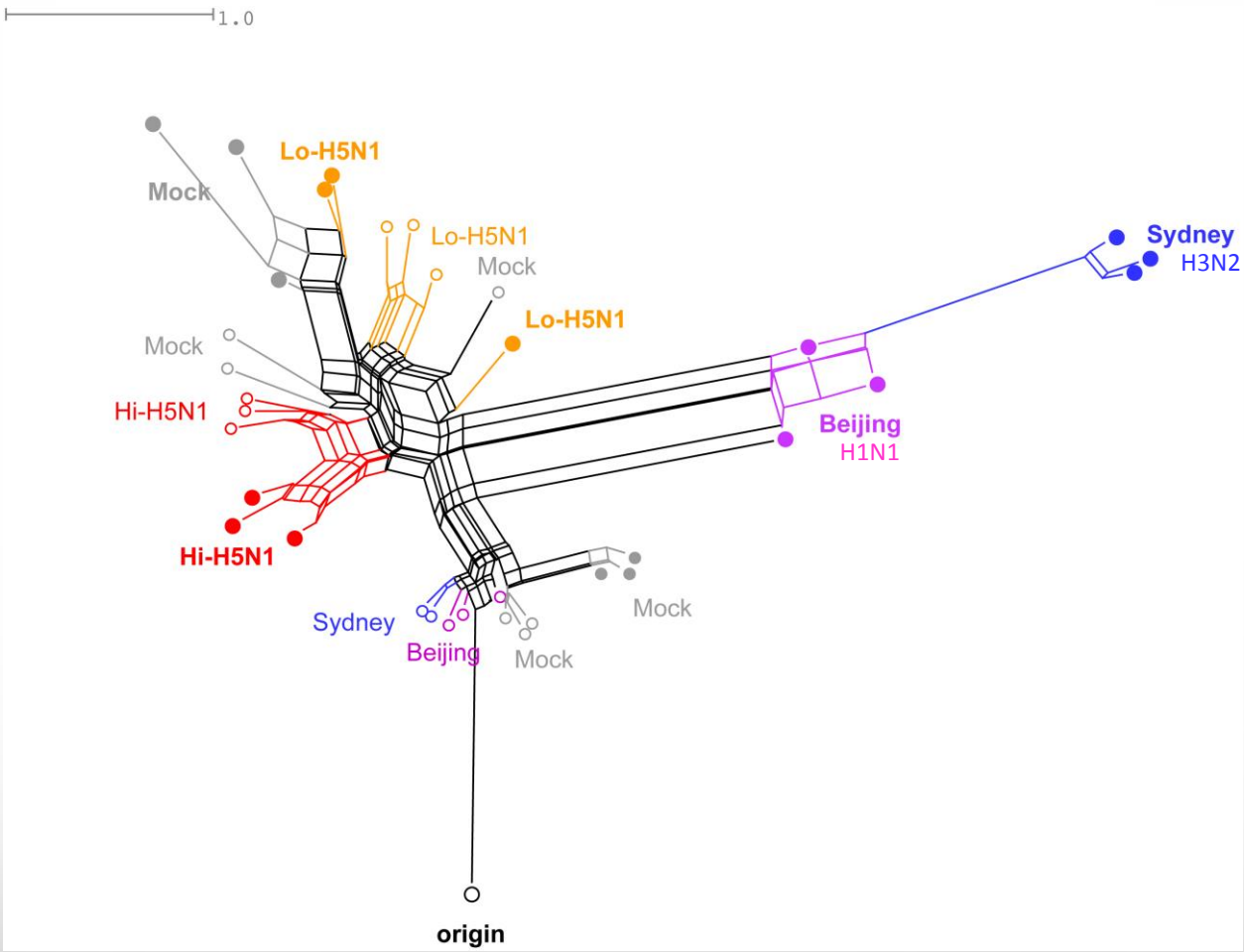
Host response as a pathogen detector

ADVANCED THERAPEUTICS AND VACCINES

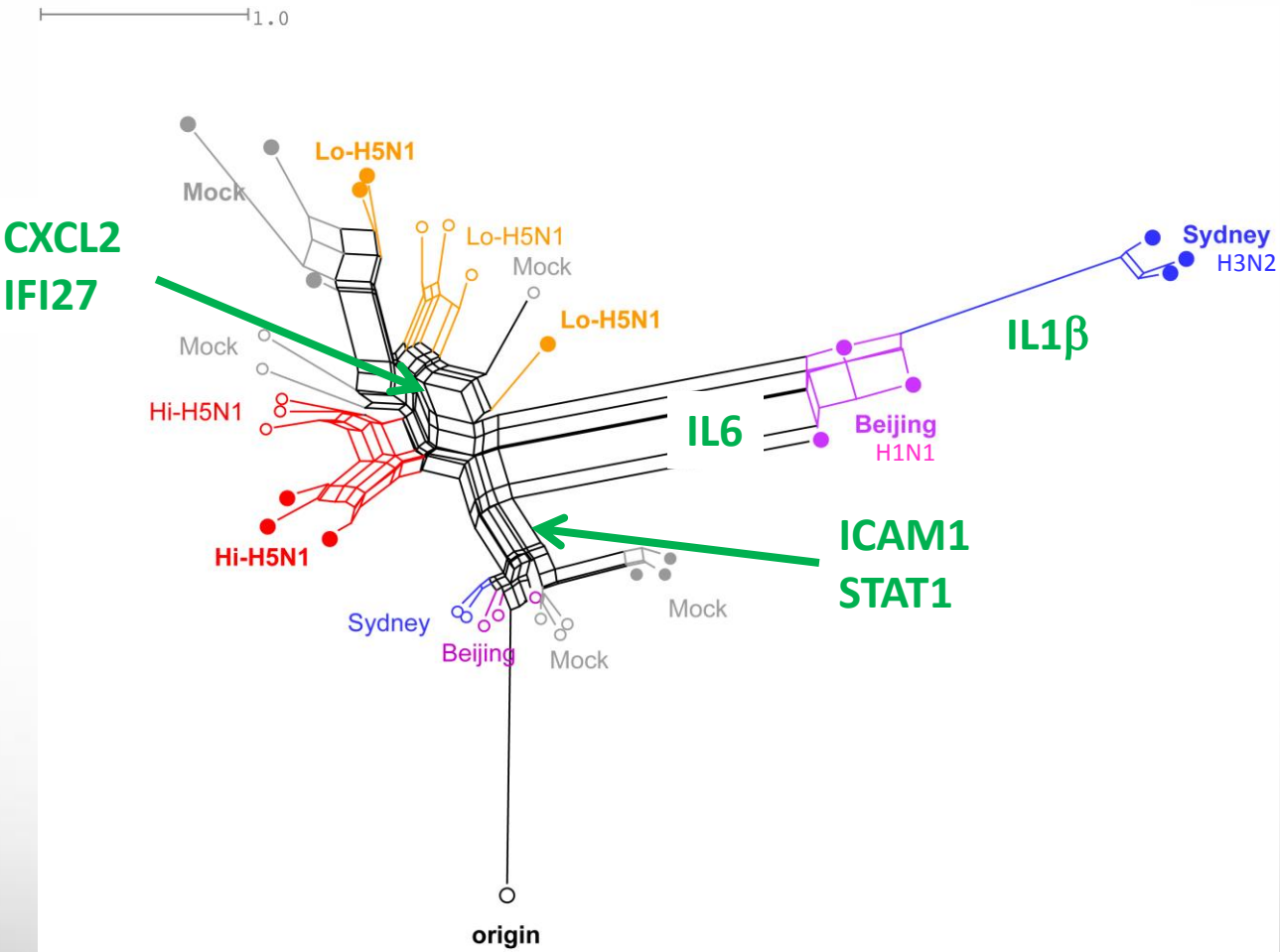
Different influenza strains will produce individual response profiles

- Primary human upper airway epithelial cells
- H5N1: A/HK/483/97 high pathogenic (HP) and A/HK/486/97 low pathogenic (LP) H5N1
- H1N1: A/Beijing/26/95; H3N2: A/Sydney/5/97
- Whole genome microarray
- 36 experiments: 4 strains, 3 time points, 3 repeats for each. 44,000 data points each experiment.

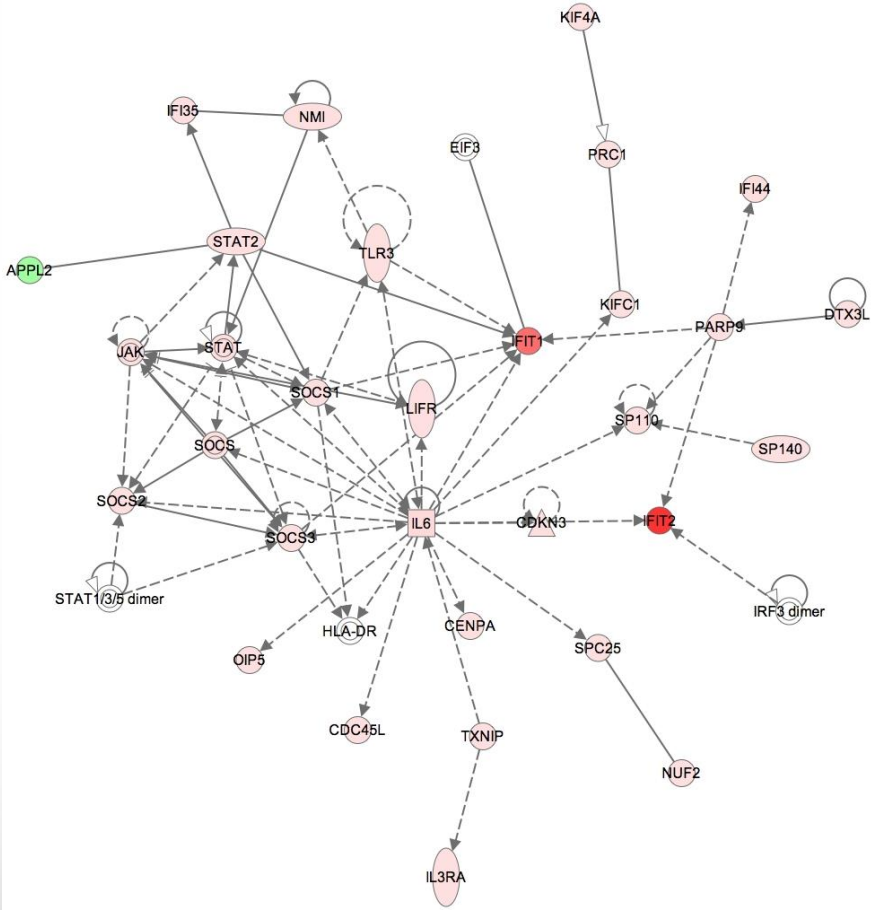
Host response as a pathogen detector

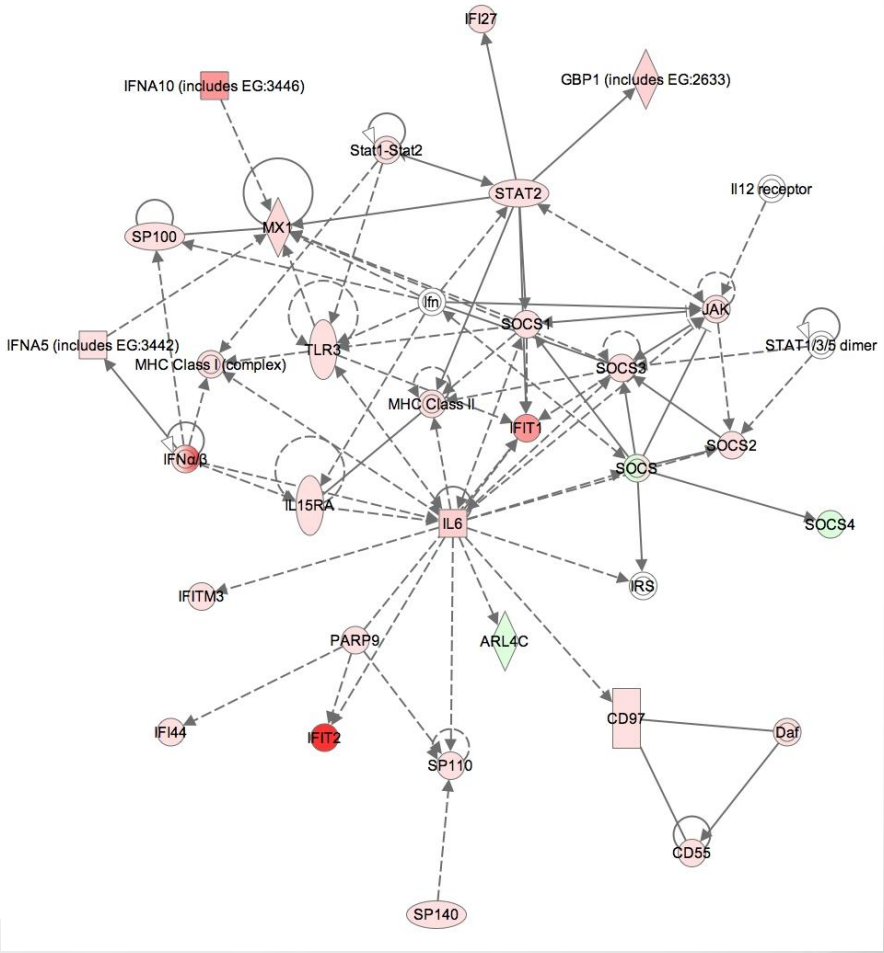


Host response as a pathogen detector



IL-6 mediated signaling network (24h)

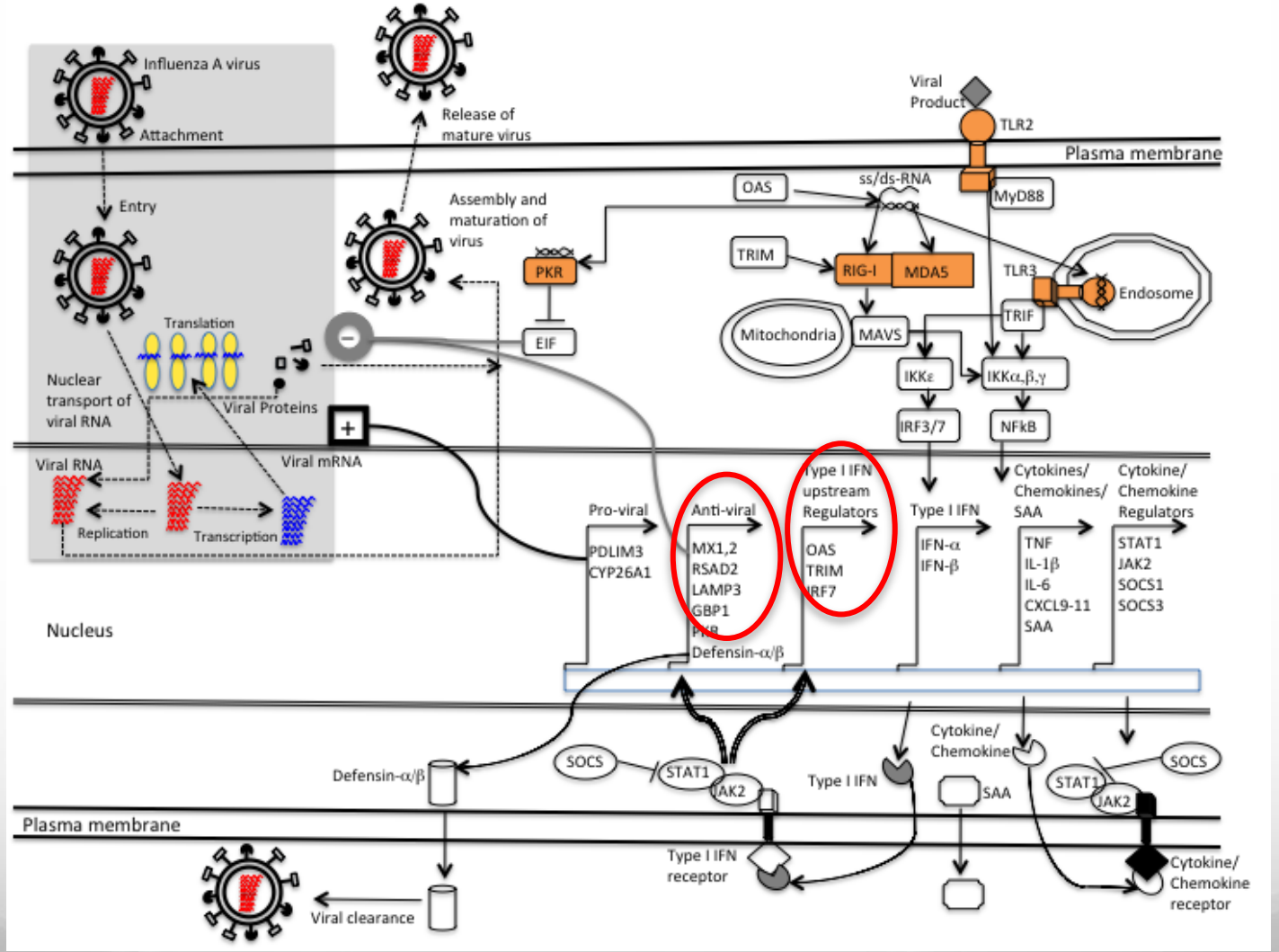




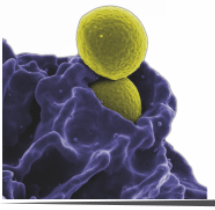
H1N1 (Beijing): Cell to cell signaling, cellular interactions, and endocytosis

H3N2 (Sydney): Antigen presentation, antimicrobial response, and cell-mediated immune responses.

Validation (e.g., viral sensors pathways)



More expressed in H3N2 than in H1N1



Modeling infection at the host scale

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Host pathogen interactions can also occur at other scales: co-infection

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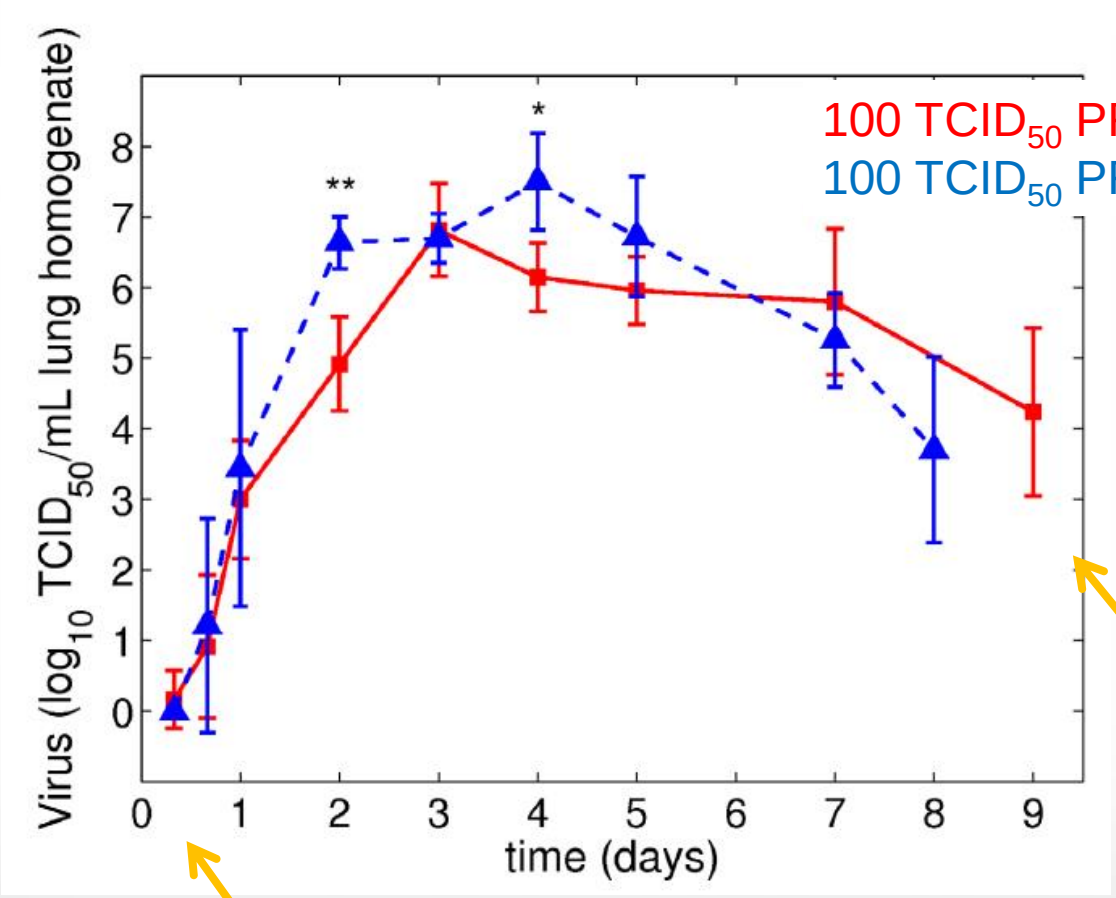
Mouse model of influenza infection



Continued decline: all survive

Intranasal infection

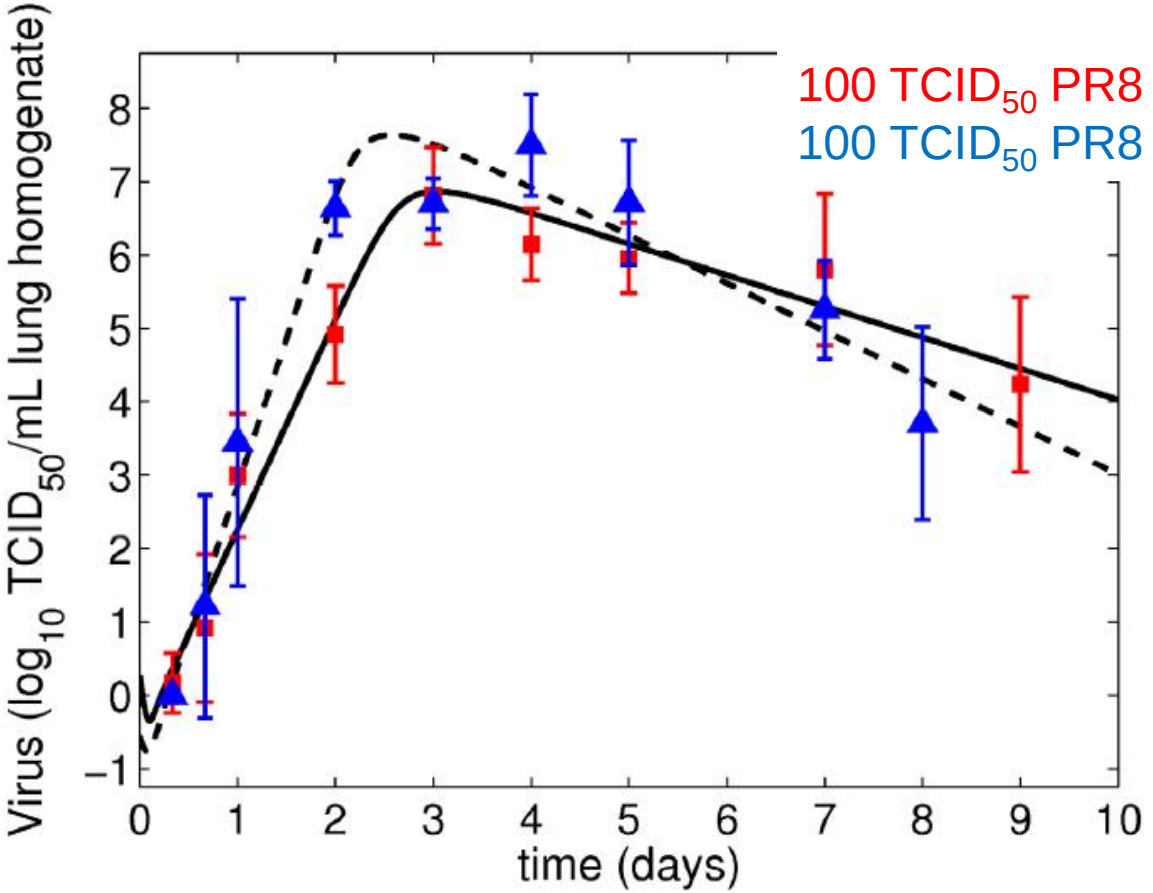
Fits to influenza infection in mice

$$\frac{dT}{dt} = -\beta TV$$

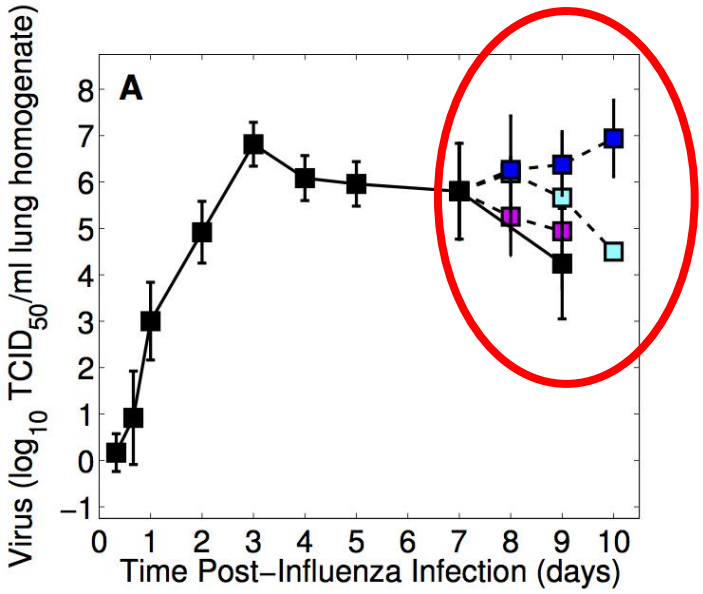
$$\frac{dI_1}{dt} = \beta TV - kI_1$$

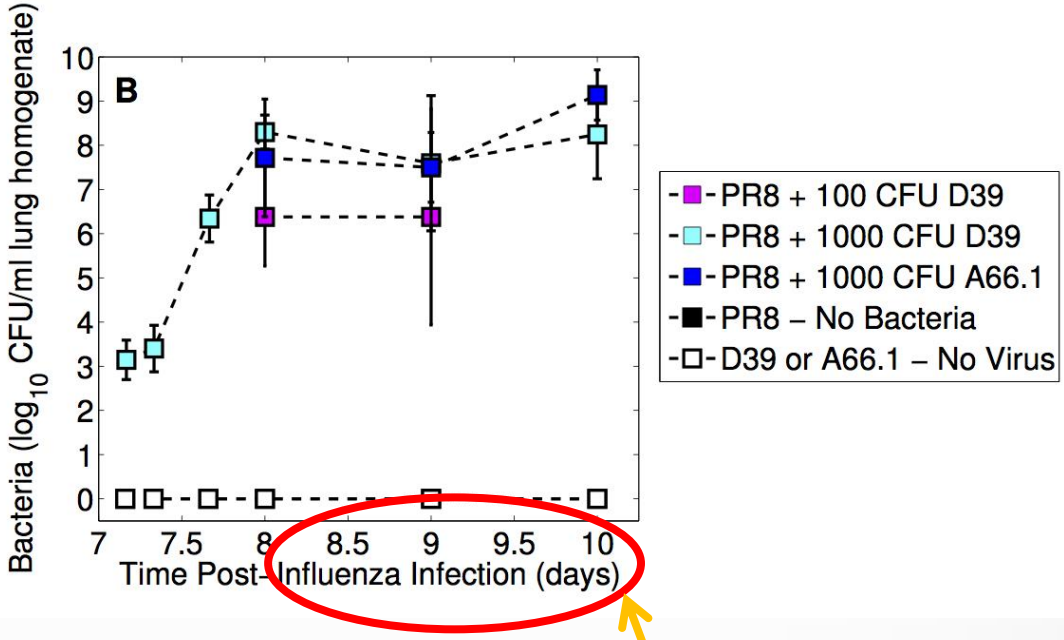
$$\frac{dI_2}{dt} = kI_1 - \delta I_2$$

$$\frac{dV}{dt} = pI_2 - cV$$



Effect of bacterial co-infection on titers

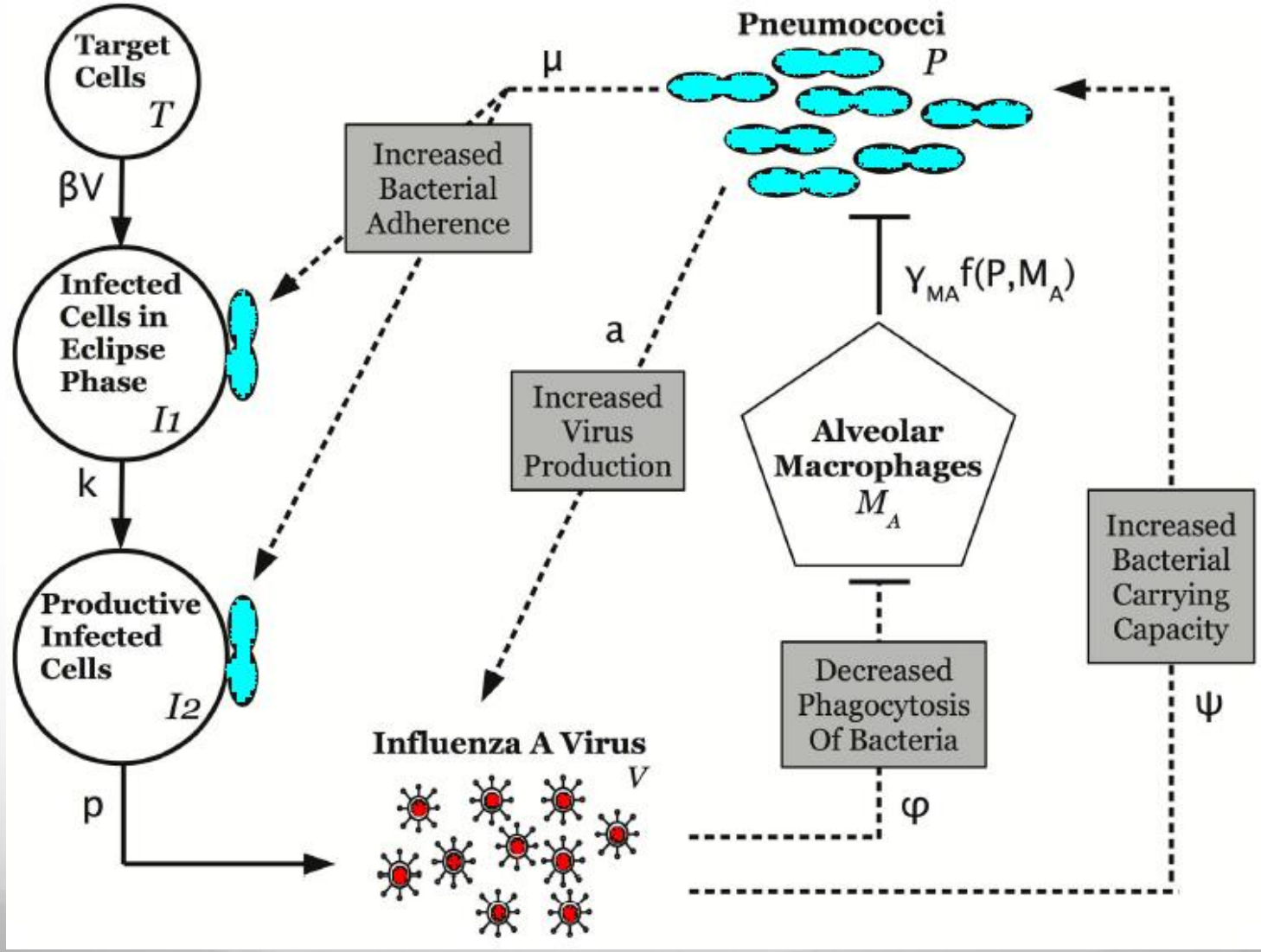




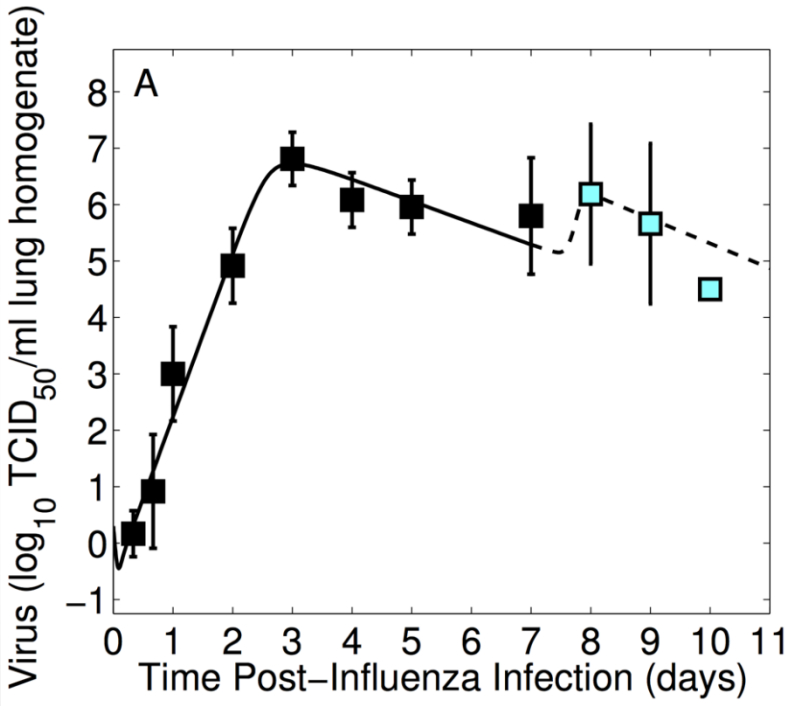
Bacterial challenge

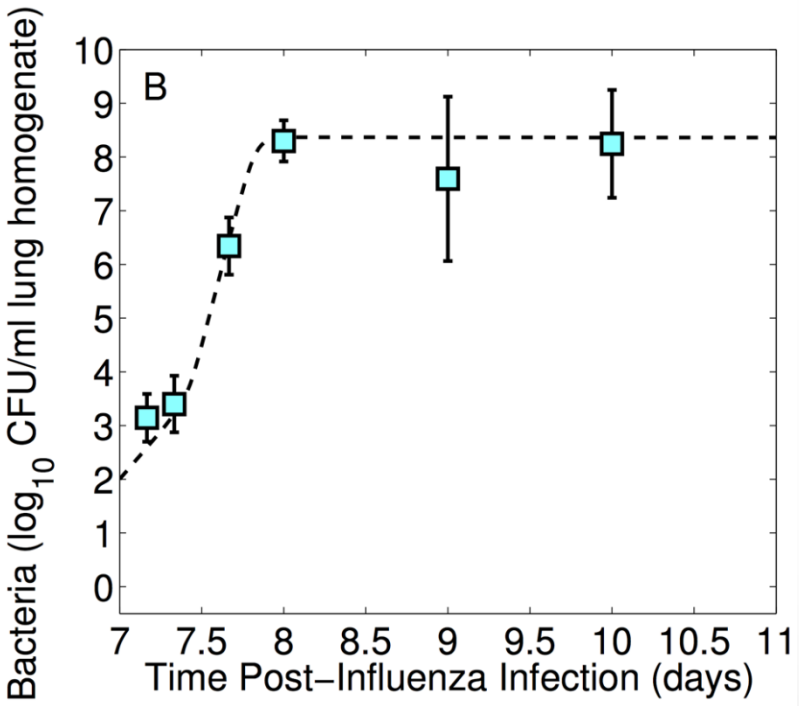
All mice euthanized

Modeling possible mechanisms



Model fits: PR8 and *S. pneumonia*



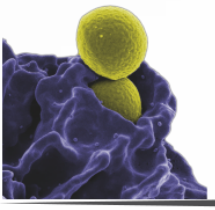


Smith *et al.*, PLoS Path. 9: e1003238 (2013)

Results

Effect	Consequences	Hypothesis
Alveolar Macrophage Dysfunction	Decreased phagocytic ability, heterogeneity in individual lung titers, and loss of phagocytic cells and early innate immune signaling	Influenza-induces phenotypic changes and/or apoptosis in alveolar macrophages
Enhanced Viral Release from Infected Cells	Rebound of viral titers and altered immune responses	Bacterial proteases and/or neuraminidases affect viral release from infected cells

Recently shown to be a factor:
Ghoneim et al. J. Immunol 2013



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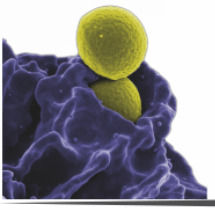
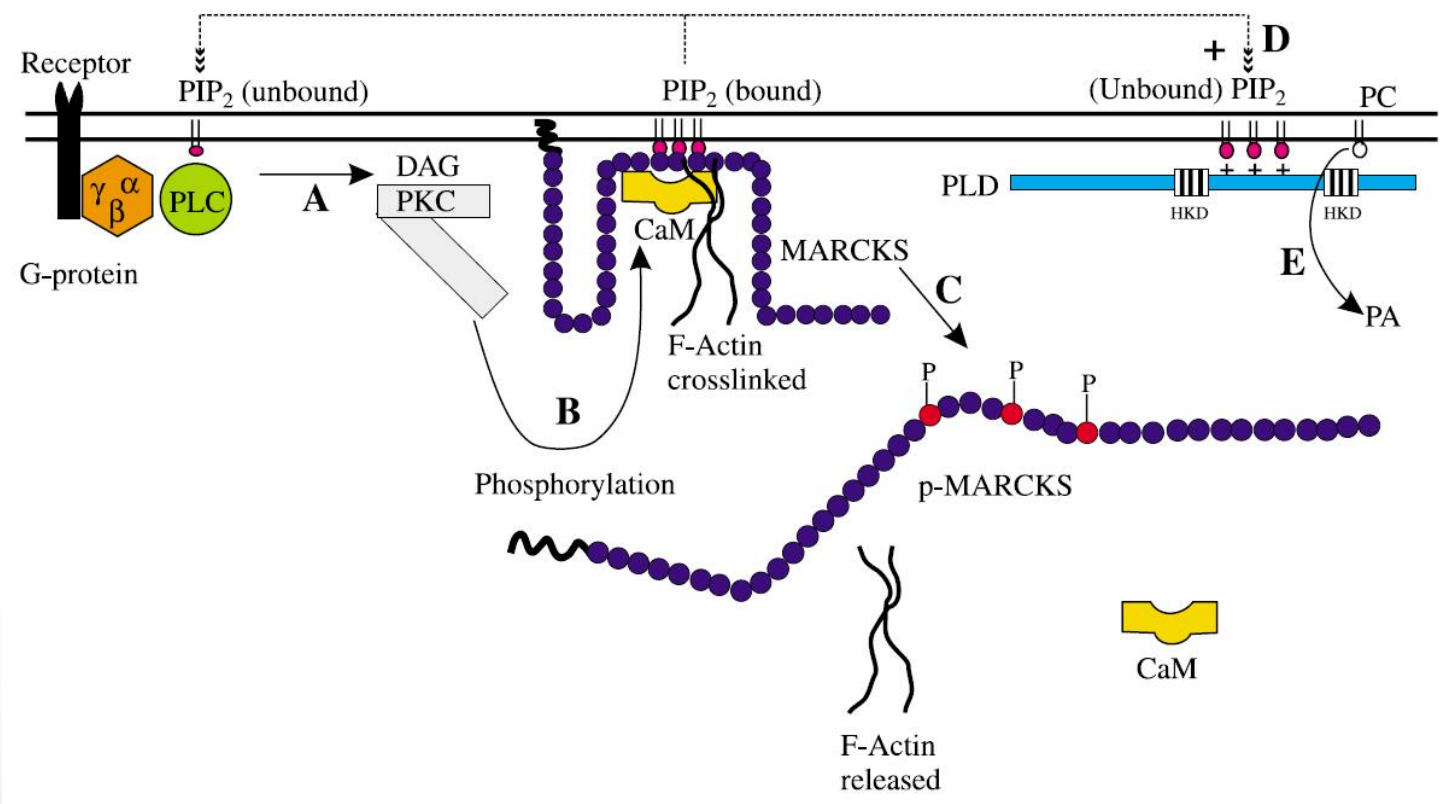


Future plans

ADVANCED THERAPEUTICS AND VACCINES

- **Approach**
  - Focus on key pathogens and key pathways
  - Multidisciplinary teams / external collaborators
  - Develop predictive models to integrate multiple data streams
- **Topics**
  - Determine how a pathogen subverts or coopts host immune pathways to propagate its lifecycle
  - Understand the immune modulatory effects of a pathogen
  - Capture host responses at the pre-symptomatic stage

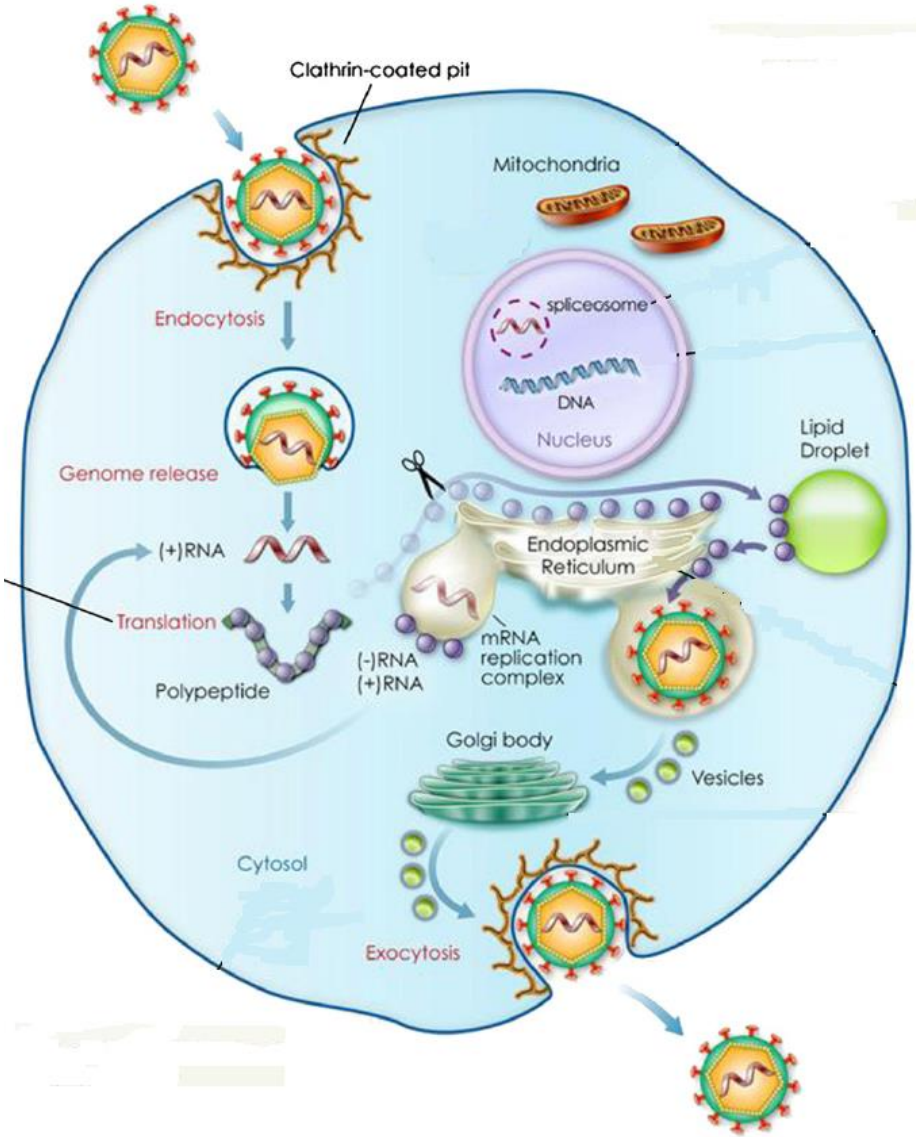
Example I - *Burkholderia*



Example II – hepatitis C virus

ADVANCED THERAPEUTICS AND VACCINES

Cyclophilin A: regulator of translation



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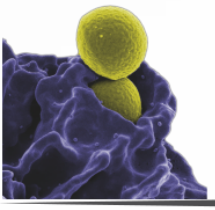

Impact

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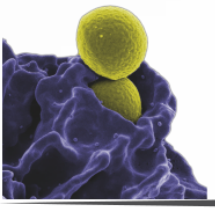

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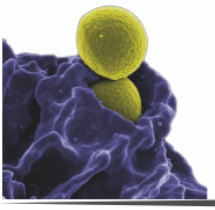

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Targeting *Burkholderia* cellular invasion